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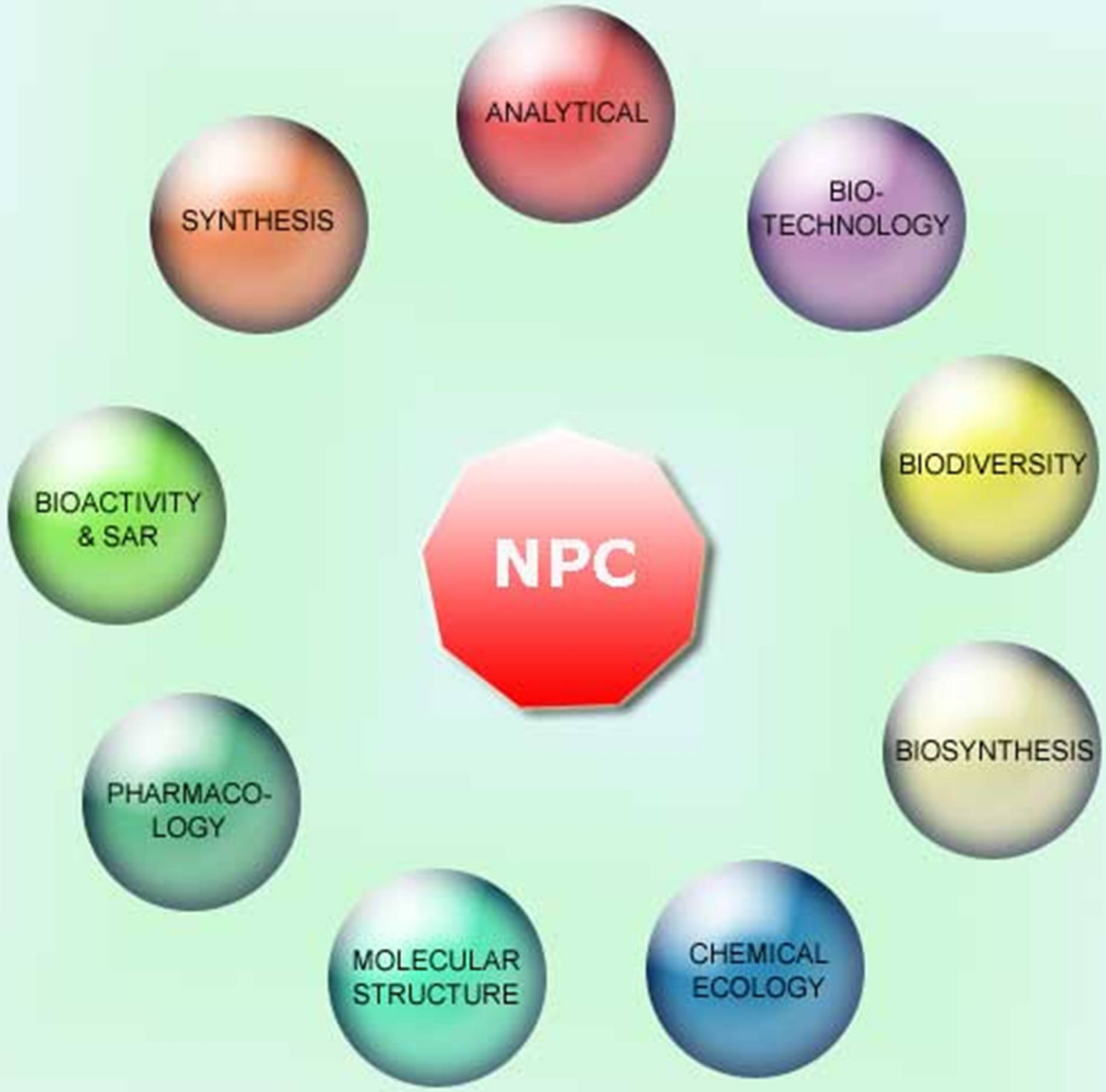


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# Genotoxic Evaluation of a Methanolic Extract of *Verbascum thapsus* using Micronucleus Test in Mouse Bone Marrow

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*Verbascum thapsus* L. is a medicinal plant and has been used to treat numerous pulmonary diseases, asthma, inflammatory disease, spasmodic coughs and migraine headaches. Several studies have demonstrated that different extracts of *V. thapsus* present antimicrobial activity. Thus, the goal of this study was to evaluate the genotoxic and cytotoxic activities of a methanolic extract of *Verbascum thapsus*, using micronucleus test in mouse bone marrow. No toxicity in bone marrow was detected in the extract-treated groups. The methanolic extract of *V. thapsus* at doses of 100, 300 and 500 mg / kg, did not produce a significant increase in the frequency of MNPCE in bone marrow and neither altered the relationship PCE / NCE respect to negative control. These cytogenotoxic findings contribute the preclinical knowledge of methanolic extract of *V. thapsus* and provide security in its use as herbal medicine.

**Keywords:** *Verbascum thapsus* L., methanolic extract, micronucleus, bone marrow, genotoxicity.

The use of medicinal plants in therapy or as dietary supplements remounts centuries ago, but it has increased substantially in the last decades [1a,1b]. The popularity of herbal medicines is related to their easy access, therapeutic efficacy, relatively low cost, and assumed absence of toxic effects.

Widespread public opinion is that being a natural product, herbal medicines are harmless and free from adverse effects. However, the safety of their use has recently been questioned due to the reports of illness and fatalities [2a-2c]. Considering the complexity of herbals in general and their inherent biological variation, it is now necessary to evaluate their safety, efficacy and quality [1b]. Thus, an assessment of their mutagenic and cytotoxic potential is necessary to ensure the relatively safe use of plant-derived medicines.

Scrophulariaceae is an important family of plants comprising over 200 genera and about 2500 species. It includes *Mimulus*, *Penstemon*, *Digitalis*, *Veronica* and *Verbascum* [3a]. Different members have been valued for their curative properties and are widely employed in domestic and regular medicine. At least 250 species of *Verbascum* are known. Among the species traditionally used in medicine, the most important is *Verbascum thapsus* L., commonly known as mullein, common mullein, great mullein [3b]. *V. thapsus* is distributed worldwide. In Argentina this species is abundant however; it is considered an exotic plant. Many studies carried out with plants collected in other countries, have shown that different extracts have antimicrobial, antitumor and cytotoxic activities [4a-4c]. Therefore, given the abundance of the species in Córdoba province, Argentina, cytotoxic and antiviral properties of methanolic extract of *V. thapsus* were investigated. The results of these previous studies have indicated that the extract markedly inhibits *Herpes suis* virus *type 1* at non cytotoxic concentrations [5a,5b]. Since this information is hopeful, it is necessary to define the cytogenotoxic potential to ensure the use of the extract at safe levels.

The aim of this study was to determine the genotoxic and cytotoxic activities of a methanolic extract of *Verbascum thapsus*, using micronucleus test in mouse bone marrow. Evaluation of micronucleus induction is the primary *in vivo* test in a battery of genotoxicity tests and is recommended by the regulatory worldwide agencies to be conducted as part of product safety assessment.

The results of micronucleus (MN) test in BALB/c mice treated with different doses of the extract are summarized in Table 1. In all cases these results are expressed as mean (± standard deviation).

**Table 1**: Mean of polychromatic erythrocytes with micronuclei (MNPCE) observed in bone marrow cell of female (F) and male (M) BALB/c mice treated with a *Verbascum thapsus* methanolic extract, and respective controls.

| Treatments | Dose mg/kg | Number of MNPCE per animal | | | | | | MNPCE (mean ± SD) | PCE/NCE (mean ± SD) |
|---|---|---|---|---|---|---|---|---|---|
| | | $F_1$ | $F_2$ | $F_3$ | $M_1$ | $M_2$ | $M_3$ | | |
| Negative control (saline) | 0 | 2 | 3 | 3 | 2 | 2 | 2 | 2.50 ± 0.55 | 1.69 ± 0.14 |
| *V. thapsus* methanolic extract | 100 | 1 | 2 | 5 | 1 | 3 | 1 | 2.17 ± 1.60 | 1.77 ± 0.12 |
| *V. thapsus* methanolic extract | 300 | 3 | 1 | 3 | 1 | 2 | 2 | 2.00 ± 0.89 | 1.79 ± 0.23 |
| *V. thapsus* methanolic extract | 500 | 5 | 1 | 1 | 2 | 1 | 2 | 2.00 ± 1.55 | 1.74 ± 0.15 |
| Positive control (cyclophophamide) | 20 | 11 | 14 | 11 | 13 | 9 | 12 | 11.7 ± 1.7* | 1.69 ± 0.10 |

Thousand cells were analyzed per animal, for a total of 6000 cells per group. SD = Standard deviation. * p< 0.001, statistically significant difference from saline group (ANOVA. Tukey`s test).

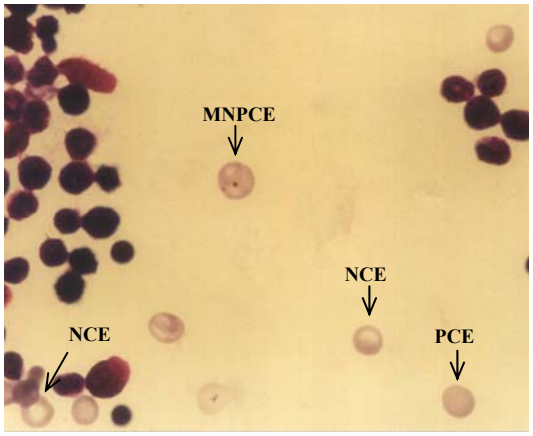


**Figure 1**: A photomicrograph of mice whole bone-marrow smear showing nucleated as well as enucleated cells (PCEs and NCEs). One the polychromatic erythrocyte also contains micronuclei.

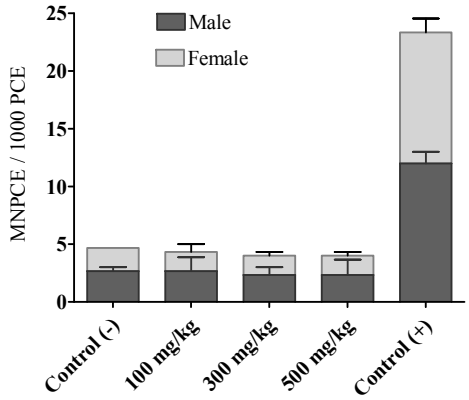


**Figure 2**: Frequency of Micronucleated Polychromatic Erythrocytes (MNPCE) induced in bone-marrow cells of female (F) and male (M) BALB/c mice treated with a *V. thapsus* methanolic extract: negative control (saline solution), positive control (cyclophosphamide 20 mg/kg body weight). Values are shown as mean ± SD.

Examples of polychromatic and normochromatic erythrocytes unaltered, normal, and the presence of micronucleated polychromatic erythrocytes are shown in Figure 1.

The percentage frequency of MN in the groups treated with 100, 300 and 500 mg / kg of methanolic extract, which were 2.17 (±1.60), 2.00 (±0.89), 2.00 (±1.55) respectively, showed no significant differences from the saline-treated group: 2.5 (±0.55). However, there was a significant increase in the frequency of micronucleus in PCE from the positive control group treated with cyclophosphamide (Figure 2).

No citotoxicity in bone marrow was detected in the extract-treated groups. Statistical analysis of the proportion PCE / NCE revealed no differences in any study group. There were no sex-dependent changes in any treatment.

*V. thapsus* methanolic extract contains iridoid glycosides (laterioside, harpagoside, ajugol, picroside IV), three iridoid ((+)-genipin, $\alpha$-gardiol and $\beta$-gardiol), one phenylethyl glycoside (verbacoside), two sesquiterpenes (buddlindeterpene A and buddlindeterpene B), one diterpene (buddlindeterpene C), and one biflavonoid (amentoflavone) [6].

Therefore, the results obtained in the present study allow concluding that the methanolic extract of *Verbascum thapsus* does not contain genotoxic and cytotoxic compounds since its administration in mice at doses of 100, 300 and 500 mg/kg, showed no evidence of genotoxicity or cytotoxicity *in vivo*. The extract did not produce a significant increase in the frequency of MNPCE in bone marrow and neither altered the relationship PCE / NCE respect to negative control.

These cytogenotoxic findings contribute the preclinical knowledge of methanolic extract of *V. thapsus* and provide security in its use as herbal medicine.

## Experimental

**Plant material and extraction:** Aerial parts of *Verbascum thapsus* L. were collected in San Luis province, Argentina. The plant material was identified by Ing. Luis A. del Vitto. A voucher specimen (N° #514) was preserved and deposited in herbal library of the "Herbario de la Universidad Nacional de San Luis, Argentina". The leaves were dried and chopped finely using a blender. Eight hundred grams of dried material were successively extracted with 3.5 L of the following solvents: n-hexane, chloroform and methanol at room temperature for 48 h. The evaporation of the extracts in vacuum at 40ºC yielded

the hexane, chloroform and methanol extracts. The methanolic extract was dissolved in saline solution and subsequently diluted to appropriate working concentrations.

**Animal's treatments:** Two months old male/female BALBc mice weighing ca. 20 g were intraperitoneally injected with a single dose of *V. thapsus* methanolic extract (volume 0.2 ml). Three doses were selected (100, 300 and 500 mg/kg) considering previous citotoxicity data obtained with Vero cells. Cyclophosphamide (Sigma) at 20 mg/kg and saline solution were used as positive and negative controls respectively.

**Mouse bone marrow micronuclei assay:** Six mice per dose were sacrificed at 24 h post-injection and femurs were removed. Femurs were prepared for the boned-marrow micronucleus test as previously described [7a]. Slides were stained with May-Grünwald and Giemsa solutions [7b] which maximized the differentiation between the polychromatic (PCE) and normochromatic (NCE) erythrocytes. To determine index of genotoxicity the number of micronucleated polychromatic erythrocytes (MNPCE) was obtained at an average of 1000 PCE, counted per animal per dose. In order to evaluate any cytotoxic effect of extract, the ratio of PCE/NCE was determined in the same sample. Statistical significance was determined by analysis of variance (ANOVA), applying software GraphPad Prism 5.0.

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**Study of Antiviral and Virucidal Activities of Aqueous Extract of *Baccharis articulata* against *Herpes suis* virus**
Cristina Vanesa Torres, María Julia Domínguez, José Luis Carbonari, María Carola Sabini, Liliana Inés Sabini and Silvia Matilde Zanon **993**

**Evaluation of Cytogenotoxic Effects of Cold Aqueous Extract from *Achyrocline satureioides* by *Allium cepa* L test**
María C. Sabini, Laura N. Cariddi, Franco M. Escobar, Romina A. Bachetti, Sonia B. Sutil, Marta S. Contigiani, Silvia M. Zanon and Liliana I. Sabini **995**

**Toxic Plants Used in Ethnoveterinary Medicine in Italy**
Lucia Viegi and Roberta Vangelisti **999**

**Diagnosis of Public Programs focused on Herbal Medicines in Brazil**
Ely Eduardo Saranz Camargo, Mary Anne Medeiros Bandeira and Anselmo Gomes de Oliveira **1001**

**Identification of Thiosildenafil in a Health Supplement**
Marcello Nicoletti **1003**

**Hypolipidemic Effect of Seed Oil of Noni (*Morinda citrifolia*)**
Diana C. Pazos, Fabiola E. Jiménez, Leticia Garduño, V. Eric López and M. Carmen Cruz **1005**

**Composition of Egyptian Nerolì Oil**
Ivana Bonaccorsi, Danilo Sciarrone, Luisa Schipilliti, Alessandra Trozzi, Hussein A. Fakhry and Giovanni Dugo **1009**

**Essential oil of *Nepeta x faassenii* Bergmans ex Stearn (*N. mussinii* Spreng. x *N. nepetella* L.): A Comparison Study**
Niko Radulović, Polina D. Blagojević, Kevin Rabbitt and Fabio de Sousa Menezes **1015**

**Chemical Composition and Biological Activity of *Salvia verbenaca* Essential Oil**
Marisa Canzoneri, Maurizio Bruno, Sergio Rosselli, Alessandra Russo, Venera Cardile, Carmen Formisano, Daniela Rigano and Felice Senatore **1023**

**Chemical Composition and Antimicrobial Activities of the Essential Oils from *Ocimum selloi* and *Hesperozygis myrtoides***
Márcia G. Martini, Humberto R. Bizzo, Davyson de L. Moreira, Paulo M. Neufeld, Simone N. Miranda, Celuta S. Alviano, Daniela S. Alviano and Suzana G. Leitão **1027**

**Chemical Composition and Antibacterial Activity of the Essential Oil of *Lantana camara var. moritziana***
Nurby Rios Tesch, Flor Mora, Luis Rojas, Tulia Díaz, Judith Velasco, Carlos Yánez, Nahile Rios, Juan Carmona and Sara Pasquale **1031**

**Activity against *Streptococcus pneumoniae* of the Essential Oil and 5-(3-Buten-1-ynyl)-2, 2'-bithienyl Isolated from *Chrysactinia mexicana* Roots**
Bárbara Missiam Mezari Guevara Campos, Anabel Torres Cirio, Verónica Mayela Rivas Galindo, Ricardo Salazar Aranda, Noemí Waksman de Torres and Luis Alejandro Pérez-López **1035**

**Antimycotic Effect of the Essential Oil of *Aloysia triphylla* against *Candida* Species Obtained from Human Pathologies**
María de las Mercedes Oliva, María Evangelina Carezzano, Mauro Nicolás Gallucci and Mirta Susana Demo **1039**

**Secretory Cavities and Volatiles of *Myrrhinium atropurpureum* Schott var. *atropurpureum* (Myrtaceae): An Endemic Species Collected in the Restingas of Rio de Janeiro, Brazil**
Cristiane Pimentel Victório, Claudio B. Moreira, Marcelo da Costa Souza, Alice Sato and Rosani do Carmo de Oliveira Arruda **1045**

**Chemical Composition and *in vitro* Antibacterial Activity of the Essential Oil of *Phthirusa adunca* from Venezuelan Andes**
Flor D. Mora, Nurby Ríos, Luis B. Rojas, Tulia Díaz, Judith Velasco, Juan Carmona A and Bladimiro Silva **1051**

# Natural Product Communications
# 2011
Volume 6, Number 7

## Contents

***Original Paper*** ***Page***

**Use of Dimethyldioxirane in the Epoxidation of the Main Constituents of the Essential Oils Obtained from *Tagetes lucida*, *Cymbopogon citratus*, *Lippia alba* and *Eucalyptus citriodora***
Luz A. Veloza, Lina M. Orozco and Juan C. Sepúlveda-Arias **925**

**Validation of the Ethnopharmacological Use of *Polygonum persicaria* for its Antifungal Properties**
Marcos Derita and Susana Zacchino **931**

**On the Isomerization of *ent*-Kaurenic Acid**
Julio Rojas, Rosa Aparicio, Thayded Villasmil, Alexis Peña and Alfredo Usubillaga **935**

**Aristolactams from Roots of *Ottonia anisum* (Piperaceae)**
André M. Marques, Leosvaldo S. M. Velozo, Davyson de L. Moreira, Elsie F. Guimarães and Maria Auxiliadora C. Kaplan **939**

**Anti-angiogenic Activity Evaluation of Secondary Metabolites from *Calycolpus moritzianus* Leaves**
Laura Lepore, Maria J. Gualtieri, Nicola Malafronte, Roberta Cotugno, Fabrizio Dal Piaz, Letizia Ambrosio, Sandro De Falco and Nunziatina De Tommasi **943**

**Chemical and Biological Activity of Leaf Extracts of *Chromolaena leivensis***
Ruben D. Torrenegra G. and Oscar E. Rodríguez A. **947**

***Citrus bergamia* Juice: Phytochemical and Technological Studies**
Patrizia Picerno, Francesca Sansone, Teresa Mencherini, Lucia Prota, Rita Patrizia Aquino, Luca Rastrelli and Maria Rosaria Lauro **951**

**Phenolic Derivatives from the Leaves of *Martinella obovata* (Bignoniaceae)**
Carolina Arevalo, Ines Ruiz, Anna Lisa Piccinelli, Luca Campone and Luca Rastrelli **957**

**Phenolic Chemical Composition of *Petroselinum crispum* Extract and Its Effect on Haemostasis**
Douglas S. A. Chaves, Flávia S. Frattani, Mariane Assafim, Ana Paula de Almeida, Russolina B. Zingali and Sônia S. Costa **961**

**Bioactivities of *Chuquiraga straminea* Sandwith**
María Elena Mendiondo, Berta E. Juárez, Catiana Zampini, María Inés Isla and Roxana Ordoñez **965**

**Free Radical Scavenging Activity, Determination of Phenolic Compounds and HPLC-DAD/ESI-MS Profile of *Campomanesia adamantium* Leaves**
Aislan C.R.F. Pascoal, Carlos Augusto Ehrenfried, Marcos N. Eberlin, Maria Élida Alves Stefanello and Marcos José Salvador **969**

**Activity of Cuban Propolis Extracts on *Leishmania amazonensis* and *Trichomonas vaginalis***
Lianet Monzote Fidalgo, Idalia Sariego Ramos, Marley García Parra, Osmany Cuesta-Rubio, Ingrid Márquez Hernández, Mercedes Campo Fernández, Anna Lisa Piccinelli and Luca Rastrelli **973**

**Antioxidant Capacity and Phenolic Content of four Myrtaceae Plants of the South of Brazil**
Marcos José Salvador, Caroline C. de Lourenço, Nathalia Luiza Andreazza, Aislan C.R.F. Pascoal and Maria Élida Alves Stefanello **977**

**Cytotoxicity of Active Ingredients Extracted from Plants of the Brazilian "Cerrado"**
Veronica CG Soares, Cibele Bonacorsi, Alana LB Andrela, Lígia V Bortoloti, Stepheny C de Campos, Fábio HR Fagundes, Márcio Piovani, Camila A Cotrim, Wagner Vilegas and Marcos H Toyama **983**

**Propagation and Conservation of Native Forest Genetic Resources of Medicinal Use by Means of *in vitro* and *ex vitro* Techniques**
Sandra Sharry, Marina Adema, María A. Basiglio Cordal, Blanca Villarreal, Noelia Nikoloff, Valentina Briones and Walter Abedini **985**

**Genotoxic Evaluation of a Methanolic Extract of *Verbascum thapsus* using Micronucleus Test in Mouse Bone Marrow**
Franco Matías Escobar, María Carola Sabini, Silvia Matilde Zanon, Laura Noelia Cariddi, Carlos Eugenio Tonn and Liliana Inés Sabini **989**

*Continued inside backcover*